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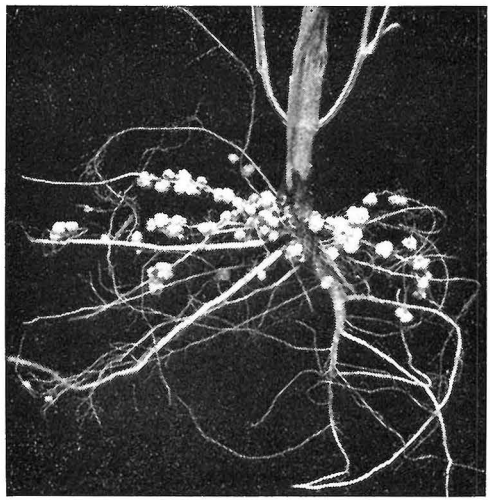
Insure Legumes With Inoculation

Harold W. Batchelor



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INOCULATE

OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

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INSURE LEGUMES WITH INOCULATION

HAROLD W. BATCHELOR

1. What is legume inoculation?

The term "inoculation" used in connection with leguminous crops refers to the bacterial culture or soil suspension applied to the seeds to insure the presence of organisms that produce nodules on roots of legumes. The term "inoculate" refers to the act of applying the material to the seed.

2. Why should legume seeds be inoculated?

Legume seeds should be inoculated to insure good nodule formation on the roots of the plants, because legumes without nodules use soil nitrogen exactly as do nonleguminous crops. Well-nodulated legumes, however, are independent of the combined nitrogen of the soil, because the nodule-forming bacteria are able to utilize the gaseous nitrogen of the air through a process known as nitrogen fixation. Part of the nitrogen thus accumulated by the bacteria in the nodules of the plant is translocated to the roots and to the tops with a resultant increase in yield of both organic matter and protein. The nitrogen left in the nodules and roots after the tops have been removed may become available for crops that follow. When unfavorable weather or soil conditions result in deficient supplies of available nitrogen, particularly during either early or late stages of growth, the effects of inoculation may be especially apparent. Well-nodulated legumes possess a rich green color and give good yields, whereas poorly nodulated plants may show a distinct yellow color and give poor yields.

3. Under what conditions should legume seeds be inoculated?

In keeping with the demands of the war effort to produce more and better quality crops, agronomists at the Ohio Agricultural Experiment Station and The Ohio State University recommend the inoculation of all leguminous seeds. The slogan is "Insure Legumes with Inoculation." For a number of years, agronomists have recommended the inoculation of sweet clover and alfalfa unless well-nodulated crops of one or the other have been grown on the field to be sown to either of these crops. This recommendation has applied especially to acid or recently limed soil (see also 18). A similar recommendation has been made for soybeans for the entire State. Red, alsike, and white clovers have been grown so generally over the State that the need for inoculating these crops has been questionable, but the wartime shortage of nitrogen warrants the inoculation of these crops as an insurance measure. The annual inoculation of canning crop peas has been considered a good practice on account of the better quality and yield of peas obtained. As common lespedeza grows rather generally throughout the southern part of the State, it has been assumed that most soils were inoculated for lespedezas. However, too frequent failures of the Korean variety indicate the need for inoculating the lespedezas (see also 20 and 21).

4. How often should inoculation be repeated?

Until the present war emergency arose, this question was answered in the following manner: "Until well-nodulated crops of any particular cross-inoculation group have been grown on a given field, it may be well to reinoculate. When one or two well-nodulated crops of one legume have been grown, the nodule bacteria will probably live in sufficient numbers from one crop to the next of the same legume to permit satisfactory inoculation of each succeeding crop. Should a period of 6 or 8 years elapse before another crop of the same cross-inoculation group is grown, it will be good insurance to reinoculate." However, with the improvements in the effectiveness of inoculation cultures and the wartime need for nitrogen, the recommendation, at least for the war period, is to inoculate all leguminous seeds every time a crop is sown.

5. What is meant by "cross-inoculation group"?

In the following table the legumes are classified into cross-inoculation groups such that the bacteria from the nodules of any legume within a given group will inoculate all the other legumes of the group but will not inoculate any legume outside its particular group. Care should be exercised to use the proper culture effective for a particular group.

The chief cross-inoculation groups of legumes

Group 1.....	Alfalfa, sweet clovers, bur clover
Group 2.....	Medium red clover, mammoth red clover, alsike clover, white clover, Ladino clover crimson clover
Group 3.....	Garden pea, field pea, vetch, broad bean, perennial pea
Group 4.....	Cowpea, common lespedeza, Korean lespedeza, partridge pea, velvet bean, lima bean (see also **20**)
Group 5.....	The beans, garden, navy, kidney, and scarlet runner
Group 6.....	Soybeans
Group 7.....	Lupine, serradella
Group 8.....	Dalea (Wood's clover)
Group 9.....	Bird'sfoot trefoil, *Lotus corniculatus*

The classification into groups has been developed as a convenience. Exceptions to it are known, and within a given group in some instances, specific strains of bacteria may be markedly superior for one legume, whereas other strains may be superior for another legume (see also **20, 21,** and **23**).

6. What is used to inoculate the seed?

Commercial legume-inoculation cultures, properly selected field soil, or macerated legume nodules can be used as a source of inoculation. (See also **10, 11, 15, 21, 22, 23,** and **24.**)

7. What commercial inoculation cultures are satisfactory?

In tests conducted at this and other Stations, the following cultures have given generally satisfactory results when favorable conditions for nodule formation have prevailed (see also **8, 10, 11, 12, 22, 24,** and **25**).

Cultures approved by the Station, 1943

(Listed in alphabetical order)

Name of culture	Type of base	Manufacturer
Hansen Humus Inoculator	Humus	Hansen Inoculator Co., Urbana, Ill.
Hansen Inoculator	Jelly	Hansen Inoculator Co., Urbana, Ill.
Kalo Inoculation	Humus	Kalo Inoculant Co., Quincy, Ill.
Legume Aid.....	Composition	Agricultural Laboratories, Columbus, Ohio
Leguminogen Inoculator.....	Humus	Leguminogen Laboratories, Delphi, Ind.
Nitragin.....	Humus	Nitragin Co., Milwaukee, Wis.
Nodogen	Humus	The Albert Dickinson Co., Chicago, Ill.
Uni-Culture	Humus	Kalo Inoculant Co., Quincy, Ill.
Urbana Culture	Jelly	Urbana Laboratories, Urbana, Ill.
Urbana Humus Inoculator	Humus	Urbana Laboratories, Urbana, Ill.
		Jobber
Blue Ribbon Inoculation	Humus	Allied Seed Co., Fort Wayne, Ind.
Ohio Farmers Brand.....	Humus	Ohio Farmers Grain & Supply Assoc., Fostoria, Ohio
Sacco Inoculant.....	Humus	Smith Agricultural Chemical Co., Columbus, Ohio

8. Are the sale and distribution of cultures controlled?

The Ohio Inoculant Law, which became effective in 1937, controls the sale and distribution of inoculation cultures in Ohio and provides for checking their quality.

9. How are seeds inoculated?

Specific directions for the use of commercial cultures are printed on the label of each container. The following are general:

Preparation of the culture suspension.—Cultures are prepared in two general forms: (a) jelly cultures, (b) humus or composition cultures. The jelly cultures consist of a firm jellylike food, on the surface of which the nodule-forming bacteria grow as a thin, slightly viscid, white scum. To remove this growth, the bottle is partially filled with water, and the growth is either rubbed off the surface of the jelly with a smooth stick or a piece of bent wire, or the stopper is again inserted into the bottle and the bottle shaken vigorously. The bacterial suspension is poured into a clean can or bucket. More water is added to the bottle, and the bottle again is shaken to remove the remainder of the bacteria. This second suspension is added to the first. It is not necessary to remove the jelly from the bottle. To the combined suspensions, enough water is added, for each bushel-sized culture, to make the volume—for small-seeded legumes, up to 1 pint, for large-seeded legumes, up to one-half pint. Cultures on humus or composition bases are poured directly into the required amount of water, mixed thoroughly, and then applied according to the directions to follow.

All culture suspensions, particularly those made in humus and composition bases and from field soil, should be shaken immediately before being applied to the seed; otherwise most of the bacteria will remain with the sediment in the bottom of the container.

Inoculation of the seed.—Legume seeds to be broadcast in the early spring can be inoculated at the barn in a bucket, bushel measure, or tub containing a small enough quantity of seed to permit its being stirred or poured from one container to another. Some farmers like to inoculate the seed on canvas on

the barn floor. Others prefer to mix it in a box with a grain scoop. A common error is to apply so much inoculum that the seeds are made wet and need to be spread out to dry. Since the bacteria are easily killed by drying, a better practice is to add the inoculum in small quantities with alternate mixing of the seeds until they are made uniformly moist but not wet. Seeds quickly absorb a slight excess of moisture and can be broadcast at once.

A peck or a half-bushel of seed can be inoculated easily in a grain sack. After a small amount of inoculum is poured onto the seed, the sack is held shut with one hand and is rolled back and forth; or while each end is grasped with one hand, the ends are raised alternately to mix the seed. More inoculum is then added if necessary, and the seed again mixed. The sack will absorb the excess moisture, and the seed can be broadcast or drilled immediately or kept moist in the sack for several hours if necessary (see also **14, 15, 16,** and **17**).

Soybeans and other large seeds can be inoculated in the drill box. If the drill box is filled about half-full of seed and a small quantity of inoculum is added, the seed can be pushed back and forth in the box to make it thoroughly moist and can then be drilled immediately. If made too moist, the seeds will lose their seed coats and even clog the drill. When it is necessary to add more seed, or if that in the box becomes noticeably dry, more inoculum can be added and mixed as before, or the seed can be inoculated in a tub and then put into the drill box. This is probably the most satisfactory method of inoculating the seed, since it prevents the bacteria from being killed by drying. It is convenient, particularly if large quantities of seed are to be drilled, because a special handling of the seed for the purpose of inoculation is unnecessary.

Humus cultures can be dusted over seed that has been moistened. Much inoculation is being applied in this way. This method was not recommended, however, until commercial cultures of this type became generally of an excellent quality. A large proportion of seed sown the last few years has been inoculated by the "dry method", that is, by dusting the unmoistened seed with the undiluted humus culture. Although this method is not generally recommended, its use prevents the spread of anthracnose in garden beans, and the method is more convenient than the others (see also **16**).

10. Where can inoculation cultures be obtained?

Inoculation cultures are usually stocked by field-seed dealers. Cultures for peas and beans are usually stocked by hardware stores or other dealers in garden seeds (see also **7, 8,** and **9**).

11. How long can inoculation cultures be kept?

Inoculation cultures are usually guaranteed for one growing season. Some are guaranteed for as little as 3 months from the date of manufacture. The expiration date of the guarantee, which appears on the label of each package, should be noted before the culture is purchased. Outdated cultures should not be used (see also **7** and **10**).

12. Does freezing injure inoculation cultures?

Under ordinary conditions, freezing probably will not injure inoculation cultures.

13. Do fertilizers injure inoculation?

Nodule formation is likely to be improved by moderate applications of fertilizer. In the case of soybean seed, germination is likely to be injured when fertilizers are drilled with the seed. However, superphosphate at normal rates of application is fairly safe. Some unsatisfactory stands of fertilized soybeans have been due to the detrimental effect of the fertilizer on the germination of the seed and not to injury of the inoculation.

14. Can unhulled seed be inoculated?

Inoculated unhulled clover seed has produced more prompt and more extensive nodulation than inoculated dehulled seed in tests conducted at the Alabama Station. A greater number of bacteria are apparently held on the rough unhulled seed than on the smooth dehulled seed. One investigator states that on account of the greater bacteria-holding capacity of the unhulled seed, from two to four times as much inoculant is required by unhulled as by dehulled seed (see also 9).

15. Can nonleguminous crops, such as corn, wheat, and oats, be inoculated?

Numerous attempts have been made to inoculate nonleguminous crops, but careful studies by experiment station investigators have failed to establish the value of such inoculation. There are no known nitrogen-fixing bacteria that are associated directly with these nonleguminous crops. Cultures intended for the inoculation of nonlegumes are not approved by the Station (see also 7).

16. Can cultures be used in a dry form on dry seed?

Some years ago in experiments conducted at Wooster to determine the effects on nodulation of using soil or commercial humus cultures with and without water, definitely better nodulation was obtained when water was used. With the better quality of cultures now on the market, less difference between the two methods might possibly be obtained. One observer has stated: "Well over half of the soybeans inoculated recently have been inoculated without the use of water. As long as the farmer continues to obtain satisfactory results with dry applications he will not risk wetting the seed regardless of instructions and warnings to the contrary." In seeding garden beans, it may be definitely advisable to inoculate by the dry method to prevent the spread of the disease known as anthracnose (see also 9).

17. Clover and alfalfa mixtures are commonly sown broadcast in the early spring. Under these conditions will inoculation on the seed be killed by direct sunlight?

Early spring sunlight has little killing effect on bacteria. There is usually sufficient moisture at the surface of the soil in the early spring to prevent killing of the bacteria by drying. Many of the seeds broadcast in the early spring fall into small soil crevices and are at least partially covered with soil.

Seedings broadcast in the summer may possibly be left uncovered for an hour or so but should be covered as soon as possible (see also 9).

18. Are the nodule bacteria and nodule formation affected by soil acidity?

With the probable exception of the lupines, which are grown largely for ornamental purposes in this country, the more nearly neutral the soil, the better will be the growth of the plant and its nodule formation. Although soybeans tolerate soil acidity better than the other legumes, their growth and nodule formation are better in neutral or slightly acid soils than in strongly acid soils. Alfalfa does not do well on soils more acid than pH 6.0.

19. Is nodule formation affected by weather and soil conditions?

Periods of dry weather may prevent nodule formation. Nodules that have been formed may subsequently slough off and decompose during periods of dry weather, according to experiments conducted at the Cornell Station. During the growing season, alternate periods of moist and dry weather may result in the production and sloughing off of several "crops" of nodules. Similarly, weather and soil conditions which result in accumulations of nitrates may prevent nodule formation. These relations should be kept in mind when observations are made on nodule formation.

20. Does Korean lespedeza require a different culture than the other lespedezas?

Although bacteria from all the legumes in the same cross-inoculation group as Korean lespedeza have been found to inoculate this crop, and bacteria from Korean lespedeza have been found to inoculate all the other legumes in the same cross-inoculation group, there is a very great difference in the relative nitrogen-gathering efficiencies of the different bacteria on the different crops; some may be highly efficient nitrogen fixers on one crop and quite inefficient or even parasitic on another. Manufacturers of inoculation cultures understand these possibilities and either produce a special culture for Korean lespedeza or a culture which contains a number of strains of bacteria that are adapted to the several plants in the lespedeza group (see also 21).

21. Are all nodule bacteria efficient nitrogen fixers?

Relatively speaking, good strains of nodule bacteria are as much more efficient nitrogen fixers than poor strains as well-adapted corn hybrids are better than open-pollinated varieties. Under controlled conditions in the greenhouse, the more efficient strains of nodule bacteria form nodules on the taproot near the seed or crown. A few nodules are formed close by on the lateral roots (see illustration on cover). Nodules formed by the inefficient strains tend to be scattered over the entire root system, with only a few on the taproot near the seed. In the field, the first season the bacteria are used the nodules of the more effective and of the less effective strains tend to form as described. In subsequent years, the bacteria become more generally distributed through the soil, and then the nodules of even the good strains are formed rather generally and uniformly over the entire root system (see also 20 and 23). The strains of nodule bacteria growing on wild legumes have been found less efficient for cultivated crops of the same cross-inoculation group than the strains especially selected and developed for cultivated crops.

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22. Are the nodule bacteria the only nitrogen-fixing bacteria?

There are at least two other groups of nitrogen-fixing bacteria, the *Azotobacter* and *Clostridium* groups. In contrast with the nodule bacteria called "symbiotic" bacteria, the *Azotobacter* and *Clostridium* groups of nitrogen fixers live in the soil with no direct relation to plants and are, therefore, called "nonsymbiotic" bacteria. It does not pay to inoculate soil with these latter bacteria, and cultures for this purpose are not approved by the Station.

23. How are commercial cultures produced?

The selection and development of efficient strains of nodule bacteria are major factors in the production of good quality commercial cultures. The surfaces of selected nodules are sterilized to remove the majority of contaminating organisms. The nodules are crushed in sterile water, and by one of several methods, the suspension is plated or streaked on a solid food or medium made especially favorable for the growth of the nodule-forming bacteria. After several days of "incubation" a single bacterium will have multiplied to many millions. These lie grouped together on the surface of the medium in small, white, glistening, slightly viscid growths or "colonies." Such colonies are transferred repeatedly to fresh solid media until the nodule-forming bacteria are completely separated from all other organisms. They are then known as "pure cultures."

Individual samples or transfers of these purified strains are then inoculated on sterilized seeds, which are put into pots or jars of sterilized sand or gravel. The pots are watered with sterile plant nutrient solution deficient in nitrogen. The pots thus prepared are kept under carefully controlled conditions to prevent contamination by other strains of nodule-forming bacteria. When a large number of such strains of organisms of the same cross-inoculation group are tested at one time on the same species of legume, marked differences occur in the appearance, yield, and nitrogen content of plants (see also 5 and 20). The bacteria that have produced the best results are used as mother-cultures for the commercial product. In the production of jelly cultures, bottles containing the nutrient jelly are inoculated from one or more of the best mother-cultures, incubated, inspected to determine the amount of growth of the bacteria and to discard contaminated cultures, packaged, and shipped to the dealer. If humus cultures are to be prepared, the bacteria are grown in large quantities, either on solid food or in aerated liquid cultures. In either case, the growth is later standardized as to bacterial count and mixed with sterilized humus or composition base, packaged, and shipped. The companies usually test representative samples of each "run" to check the whole manufacturing process. The production of cultures has thus become a carefully controlled and well-standardized process (see also 7, 8, 10, 11, 15, 20, and 26).

24. Are seed treatments, disinfectants, and inoculation cultures the same?

The inoculation cultures for legumes should not be confused with various chemical preparations used as seed treatments to control plant diseases. Formaldehyde dust, copper, mercury, or zinc compounds are used on various seeds to control diseases. These are valuable products for the purposes for which they are intended but are not inoculation cultures. Inoculated seed should not be allowed to come in contact with seed treatments or disinfectants.

25. How much moisture should humus and composition cultures contain?

It is not practical to set a minimum moisture standard for all cultures, because the bases vary in their water-holding capacity. Satisfactory cultures usually feel moist but not wet.

26. How are commercial cultures tested?

Pot tests in the greenhouse, such as described under **23**, are made. Although field tests would be more desirable, too many difficulties in their control make routine field tests unsatisfactory and expensive. In addition to the pot tests, the numbers of bacteria, the degree of acidity or alkalinity, and the moisture content of the culture are determined (see also **7**, **8**, and **23**).

27. How should hay- or meadow-seed mixtures be inoculated?

Hay- and meadow-seed mixtures can be prepared and then inoculated in the usual manner. It is not necessary to inoculate the individual species of seed separately before the different seeds are mixed. New multiple-type cultures which contain bacteria for two or more cross-inoculation groups, for example, the alfalfa-sweet clover group and the red-alsike-white clover group, have been developed especially for this purpose. Some of the latter cultures also contain bacteria for soybeans. Parts of such cultures left over from inoculating the early spring hay- and meadow-seed mixtures should not be held over for soybean inoculation to be used several months later, because the soybean bacteria will probably be killed by excessive drying. New soybean cultures should be used.

28. Should seed left over from the previous year be reinoculated before sowing?

Yes, because the inoculation bacteria are rather easily killed by excessive drying. All seed should be sown as soon as possible after it is inoculated. For early spring seedings when temperatures are low and humidities rather high, inoculated seed can probably be kept a week or two before seeding if it is sacked and stored in an unheated building. This practice is not recommended, however.

Additional information can be obtained from—

Microbiologist,
Ohio Agricultural Experiment Station
Wooster, Ohio

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